Field Tests With an Aerial Application of Bacillus thuringiensis

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CONTENTS

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INTRODUCTION

Bacillus thuringiensis var. *thuringiensis* Berliner was used experimentally in 1963 against larvae of the gypsy moth, *Porthetria dispar* (L.), in a Connecticut State Forest. The test was one of several made by entomologists in the Northeast.

A new formulation of *B. thuringiensis,* Thuricide 90T, was expected to make possible accurate application from the air. In the past, only wettable powder formulations of *B. thuringiensis* were available, and in airplane application against the gypsy moth these gave variable results. Wettable powders tended to settle rapidly, and an even application was difficult to obtain. Moreover, it was impossible to apply the wettable powder spray at the high concentrations needed for control of the gypsy moth, because of the high amount of solids present in the finished spray.

The test area was known to have an infestation of gypsy moths as well as a considerable population of various geometrids. The geometrids were principally the linden looper, *Erannis tilaria* (Harr.) and *Phigalia titea* (Cram.). Smaller numbers of an oak leaf roller, *Argyrotoza semipurpurana* Kft., and an unidentified geometrid larva were present as well as a scattering of species from other lepidopterous families. This gave the opportunity to field test the new formulation of the *Bacillus* against a variety of deciduous-forest insects.

APPLICATION METHODS AND MATERIALS

The test plots were located in Portland, Connecticut, in the Meshomasic State Forest. The area is typical of the cutover deciduous woodland dominated by oaks that covers much of Connecticut. One of the permanent forestry plots maintained by the Connecticut Agricultural Experiment Station is nearby. The vegetation in this forestry plot, described by Collins (1962), is similar to that of the test plots.

There were six 50-acre plots, each within 1.2 miles of all others. With the exception of Plot 1, differences in elevation within any plot did not exceed 150 feet and all were on a western exposure. Two additional check plots (Plots 7 and 8) were established near those treated areas farthest from Check Plot 1 (Figure 1).

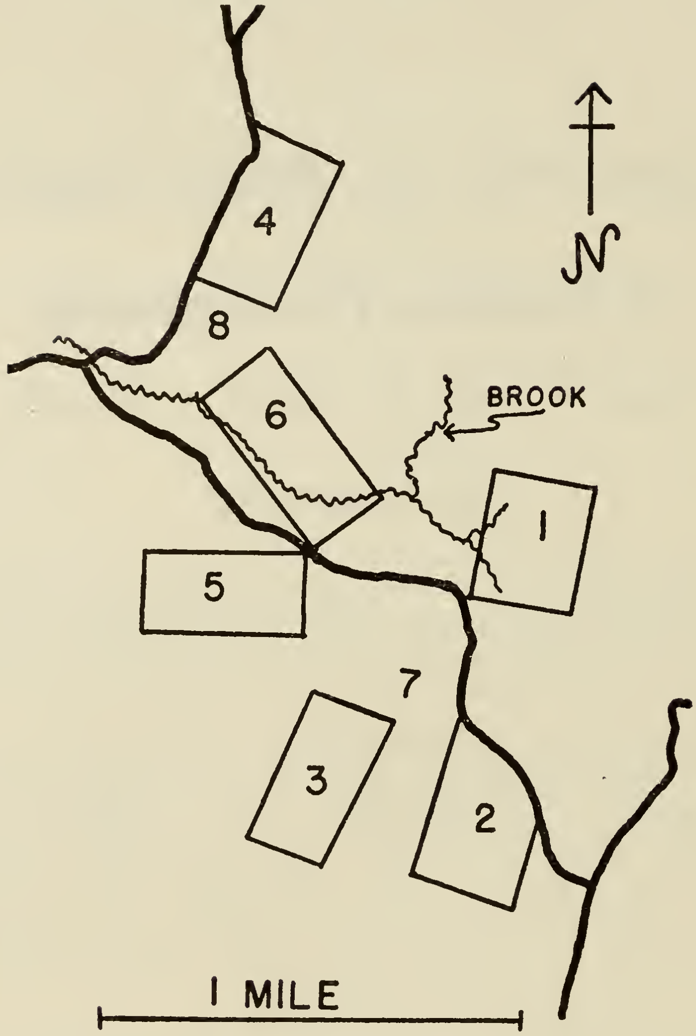

Figure 1. Map of test area.

The spray was applied by a Bell helicopter used for commercial work, and the sprayer was thoroughly cleaned before use. Thuricide 90T was applied at rates of ¼, ½, and 1 gallon of concentrate with enough water to deliver 2 gallons of finished spray per acre. The sprayer on the helicopter was set to deliver 1 gallon per acre. Thus the spray was applied in two loads of 50 gallons each over the whole 50-acre plot. This also minimized the danger of miss and increased the coverage. Two plots were sprayed with 1 gallon of Thuricide 90T per acre (Plots 2 and 6), two plots were sprayed with ½ gallon of Thuricide 90T concentrate per acre (Plots 3 and 4), and one plot was sprayed with ¼ gallon of Thuricide per acre (Plot 5).

Each plot was marked at the corners with a weather balloon and/or smaller yellow-orange balloons filled with helium and elevated through the tree canopy on long strings. Before each plot was sprayed, the pilot flew over it to familiarize himself with the topography of the area and location of the markers.

No attempt was made to measure the exact coverage, but observers were in each plot at spraying time to check on conditions of the application. (Recent work on an aerial forest spray project by Maksymiuk (1963a, 1963b) has shown the difficulty in obtaining an accurate measure of the foliage spray deposit by use of cards or plates on the ground.)

Plot 2 was sprayed on May 14, and about one hour later a heavy rain occurred. The other plots were sprayed on May 15.

SAMPLING METHODS

Before the gypsy moth larvae had emerged from the egg masses in the spring, an experienced crew of gypsy moth scouts made counts in all plots of the number of egg masses per acre at 12 different points within each plot. In August after the new egg masses were deposited, the same men made further counts within each plot and in areas adjacent to them.

The numbers of lepidopterous larvae present in each plot were also counted before the spray was applied. In order to obtain an accurate sample in the plots, four men individually counted the number of larvae on 20 leaves each of beech and birch and 20 clusters composed of five leaves of oak at six different spots in each plot. Thus the larvae on 3360 leaves were counted from 24 different spots and three tree types in each plot. These counts were repeated in June, but at this time 20 leaves were counted from each oak tree at each sampling point making a total of 1440 leaves per plot.

Within each plot, 12 cloth drop nets of one square yard each were suspended by four corners to catch dead insects and frass falling from insects feeding in the trees. These nets were placed in two rows of six nets extending in two parallel lines across the center two thirds of a plot. However, only six nets were placed in Plots 7 and 8.

Defoliation estimates were made in June by four individuals who independently evaluated the same 24 trees in each plot. The selection of the trees was restricted to upper-story oaks but was otherwise at random. An additional reading was taken on the eastern side of Plot 2 where an obvious difference due to treatment could be seen. A further estimate of defoliation was measured by the amount of light coming through the tree canopy as recorded by a Weston light meter.

RESULTS

Weather Conditions During Test Period

Period	*Temperature*	
	Max.	*Min.*
May 1 to May 10	69.7	47.3
May 11 to May 20	66.2	43.9
May 21 to May 30	65.8	42.6

These data are averaged from daily readings taken at the nearby Middletown weather station. The early, warm temperatures during the first 10 days in May hurried the seasonal development of both plants and animals. The loopers were developing particularly fast, and their feeding damage made it necessary to treat before they stripped the young leaves. Around the 10th, the leaves of the beech and birch were getting close to full size. Oak leaves were not quite half developed. The density of the infestation in some areas resulted in defoliation of the lower limbs of oak by the 10th of May. After the 10th of May, the weather became cooler for the remainder of the month. This reversal of the usual weather pattern for May was not suitable for normal development of the loopers or gypsy moth, and there was an increasing number of days when the larvae were unable to feed properly. From May 18 to May 22 there were 5 days of intermittent showers along with cool weather that had a further effect on the growth of the larvae.

Gypsy Moth Egg Mass Counts

Pretreatment counts, as expected, showed a considerable variation in the number of egg masses throughout any one plot, but the egg mass counts in every plot gave indication of the presence of a considerable population of gypsy moth (Table 1). According to counts, the infestation gradually tapered off toward the northern part of the test area.

Table 1. Gypsy moth egg masses per acre before and after application of *B. thuringiensis* sprays

Dosage rate per acre	Pretreatment		Postreatment			
			Treated		Untreated[1]	
	Ave.	Range	Ave.	Range	Ave.	Range
¼ gal. (Plot 5)	287	112-1172	97	32-240	..	......
½ gal. (Plot 3)	465	128-1968	41	16-80	35	16-48
½ gal. (Plot 4)	159	32-224	140	32-592	75	32-128
1 gal. (Plot 2)	605	272-992	33	16-48	38	16-64
1 gal. (Plot 6)	275	160-432	91	32-160	87	32-176
Check (Plot 1)	355	176-912	...	......	40	0-96

[1] Untreated areas outside of the listed plot.

The postreatment count of gypsy moth egg masses showed a marked reduction in all plots whether they were treated or not. Counts made both outside and inside several of the treated plots showed no differences in numbers of egg masses as a result of spraying with various concentrations of *B. thuringiensis,* nor did the dosage rate have any relation to the amount of reduction between plots. The size of the individual egg masses also did not show any discernible response to the treatment applied.

Leaf Sampling of Larvae

Pretreatment count on leaves

Shortly before spraying, the larval population was measured in all plots. The gypsy moth larval population was remarkably uniform (Table 2) and to a lesser degree the geometrid population was also (Table 3). This was true

Table 2. Average number of gypsy moth larvae per 20 leaves before and 3 weeks after treatment

Dosage rate per acre	Host tree					
	Oak		Beech		Birch	
	Before	After	Before	After	Before	After
¼ gal. (Plot 5)	3	0.5	3	0.1	1.8	0.1
½ gal. (Plot 3)	3	1.0	4	0.8	0.5	0.04
½ gal. (Plot 4)	4	0.9	3	0.2	0.2	0.1
1 gal. (Plot 2)	3	0.3	3	0.1	0.8	0
1 gal. (Plot 6)	4	0.7	4	0.2	0.1	0.1
Check (Plot 1)	4	1.6	3	1.0	1.3	0.3
Check (Plot 7)	..	2.6	..	1.0	..	0.6
Check (Plot 8)	..	0.6	..	1.3	..	0

Table 3. Average number of geometrid larvae per 20 leaves before and 3 weeks after treatment

Dosage rate per acre	Host tree: Oak Before	Oak After	Beech Before	Beech After	Birch Before	Birch After
¼ gal. (Plot 5)	10	1.5	23	0.3	21	0.4
½ gal. (Plot 3)	8	1.9	22	0.4	17	0.4
½ gal. (Plot 4)	15	3.9	23	0.4	14	1.5
1 gal. (Plot 2)	8	1.6	20	0.1	16	0.3
1 gal. (Plot 6)	12	2.8	17	0.4	11	0.2
Check (Plot 1)	15	4.1	24	1.2	9	1.8
Check (Plot 7)	..	5.6	..	0.3	..	0.6
Check (Plot 8)	..	9.1	..	0.5	..	2.0

not only among plots but also among sampling points within a plot and among persons doing the sampling. This uniformity however did not include comparisons between host trees. In Tables 2 and 3 only the mean number of larvae per sampling unit (20 leaves) is given although 24 spots within a plot were sampled for each of the three types (oak, beech, birch) noted. In these pretreatment counts, the oak was sampled as units of 20 clusters, with five leaves per cluster. However in Tables 2 and 3 this has been converted to the equivalent of 20 leaves so direct comparisons may be made between sampling dates and host trees.

The number of egg masses per acre (Table 1) did not necessarily correspond with the actual larval population found on the leaves just before spraying (Table 2). Egg parasites, winter kill, and dispersion of first instar larvae apparently created a relatively even distribution pattern over all the plots.

In pretreatment counts, the geometrid larvae were 98 per cent linden looper (*Erannis tilaria*) and 2 per cent *Phigalia titea* on all three host types sampled. This proportion was to change as the season advanced. Spring cankerworms (*Paleacrita vernata*) formed a negligible portion of the population even though numerous males were noted earlier.

Postreatment count on leaves

On June 5, 3 weeks after the sprays were applied, counts of living loopers and gypsy moth larvae were again made on leaves of oak, beech, and birch. These results are shown in Tables 2 and 3.

In the pretreatment counts, the populations on beech and birch were high. Soon after this the number of loopers feeding on the leaves of birch and beech fell to a low level, as seen from the postreatment counts of June 5. It was evident from the shot-hole appearance of these leaves that this feeding had taken place when the leaves were small and had then largely ceased. The looper larvae may have migrated from birch and beech, possibly to more suitable host trees or undergrowth. It was noted that regrowth chestnut was common in the plots. Loopers were uncommon on chestnut up to the middle of May but later heavily defoliated this species.

By June 5 the loopers were in the last instars. Although Table 3 indicates a reduced population of geometrids, at these stages a few larvae per 20 leaves can result in heavy defoliation. The numbers of looper larvae on oak were higher in all control plots than in the treated plots, but there were differences between treated plots that are not accountable to the dose of *B. thuringiensis*

used. The geometrids maintained their same relative numbers in all plots (a reduction to about one-fourth) with the exception of the most lightly treated plot (Plot 5). Despite the treatment it appeared that Plots 4 and 6 had a higher population than the plots treated like them, Plots 3 and 2.

As the season advanced the percentage of linden looper in the total complex of lepidopterous larvae on oak steadily declined from 89 per cent in mid-May to 72 per cent in late May to 45 per cent in early June. This decline which was also seen on beech and birch was probably caused by disease. Although Table 3 shows little correlation between dosage rate and geometrid survival, the proportion of linden looper within each plot did show a decline with increased dosage whereas *Phigalia titea* apparently did not.

The gypsy moth larvae, despite a uniform pretreatment population, did not show a response directly correlated with the treatment used (Table 2). The check plots generally had higher populations than the treated, but, as with the geometrids, the most lightly treated plot (Plot 5) showed one of the greatest reductions. The other plots showed a response more nearly in line with the dosage rate.

If the young gypsy moth larvae rejected the treated leaves or otherwise were slower in feeding in the treated plots, it would be expected to show in a slower rate of growth. Figure 2 demonstrates that larval growth, as measured by head capsule width, was similar in both the most heavily treated plots and the nearest check plots.

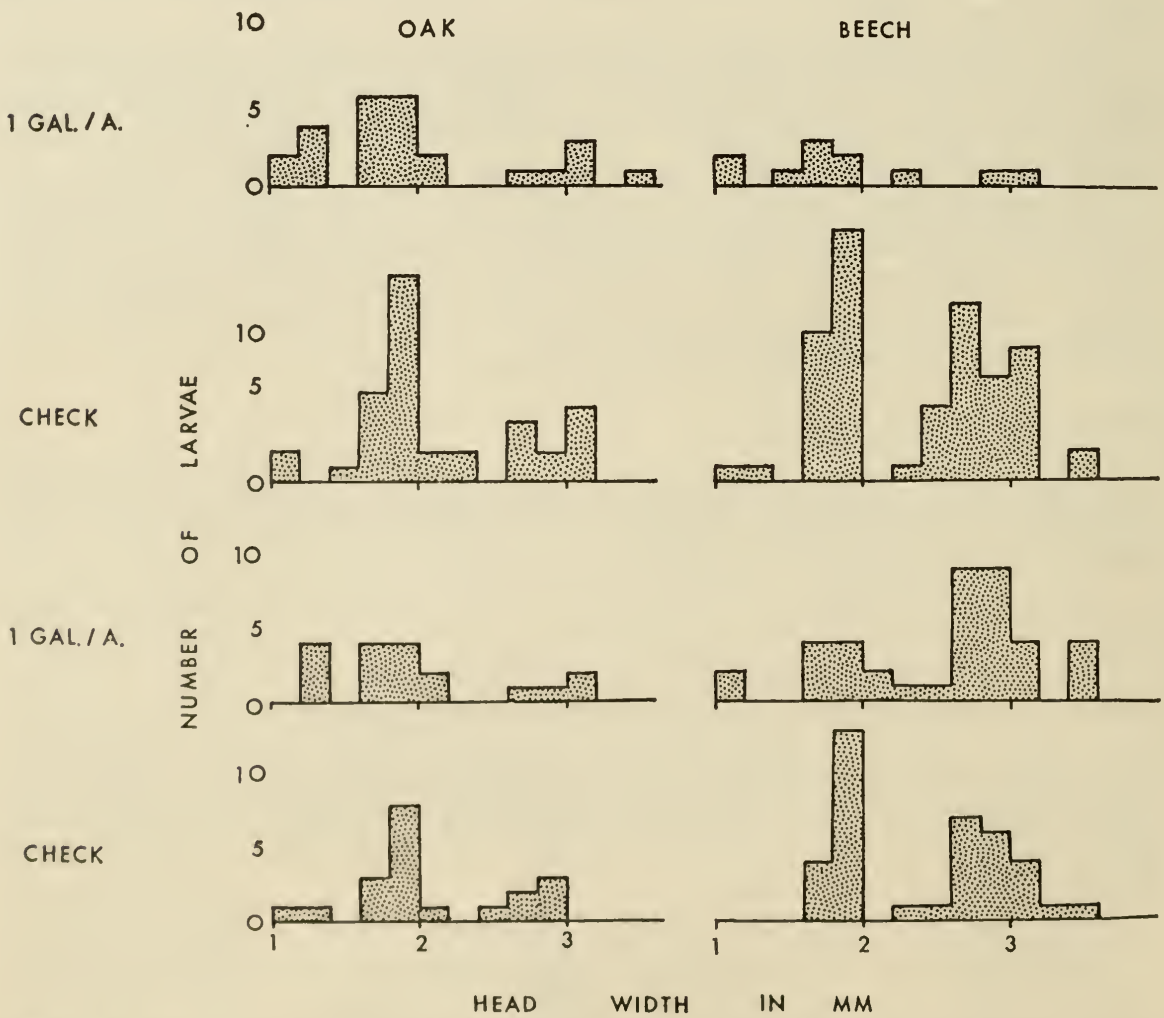

Figure 2. Comparative head widths of gypsy moth larvae on oak and beech in plots treated with 1 gallon per acre of *B. thuringiensis* and in nearest checks.

Drop Net Samples

Larvae

Collections of dead and paralyzed larvae falling from the trees into the drop nets were made on seven different days. Plot 2 was sprayed on May 14, the day before the other plots were treated. A sample was taken on May 15 and an average of six nets yielded 69 loopers and 4 gypsy moth larvae that were dead or paralyzed. The remainder of the data are found in Tables 4 and 5. It should be remembered that Plot 2 is one day older than the other plots.

Table 4. Dead or paralyzed geometrid larvae collected in drop nets following treatment, expressed as an average yield for six nets

Dosage rate per acre	Date of Collection					
	May 16	May 17	May 20	May 21	May 24	May 27
¼ gal. (Plot 5)	82	69	113	[a]	70	23
½ gal. (Plot 3)	88	90	238	87	117	42
½ gal. (Plot 4)	132	65	113	44	59	31
1 gal. (Plot 2)[b]	59	83	167	68	52	22
1 gal. (Plot 6)	96	92	112	58	87	35
Check (Plot 1)	29	21	[c]	29	28	22
Check (Plot 7)	21	19	13	14	31	21
Check (Plot 8)	5	18	[c]	26	24	17

[a] Nets cleared.
[b] Plot 2 was sprayed one day before other plots — each reading is one day more from treatment.
[c] No collections were made in Check (Plot 1) and Check (Plot 8) on May 20. This was done the following day so this is an accumulative sample not to be compared to remainder of collection on May 21.

As would be expected from other studies (Quinton and Doane 1962) the linden looper and other species of loopers were quick to respond to the effects of *B. thuringiensis.* One day after treatment a considerable amount of mortality was evident in the treated plots. Mortality differences between larvae in treated and untreated woods were greatest by the fifth day. On May 20 the accumulation of dead and paralyzed larvae from May 17 was taken. Two of the checks were not read until the following day but the mortality of looper larvae in all of the treated plots was well above the 4-day accumulation in these check plots. This should be kept in mind when examining the collection data for May 21, the sixth day from treatment. Here only Check Plot 1 is useful for comparison with the treated plots. Mortality due to treatment remained evident until May 24. No difference in looper mortality was evident on May 27. This was expected since the residual effect of *B. thuringiensis* is considered to be approximately one week.

The high number of loopers in the plots made possible clear differences between the number of dead and paralyzed larvae falling into drop nets in treated and untreated woods. The initial gypsy moth infestation, however, was not high, and so the possible number of gypsy moth larvae that could be caught by the nets was proportionately low. On May 20, 5 days after application (6 days for Plot 2) there appeared to be some mortality of gypsy moth larvae due to treatment. Although the total number of gypsy moths was not high, the numbers killed were in direct proportion to the dosage of *Bacillus* applied (Table 5).

Table 5. Dead or paralyzed gypsy moth larvae collected in drop nets following treatment, expressed as an average yield for six nets

Dosage rate	Date of Collection					
per acre	May 16	May 17	May 20	May 21	May 24	May 27
¼ gal. (Plot 5)	2	2	7	[a]	8	6
½ gal. (Plot 3)	5	5	7	12	11	8
½ gal. (Plot 4)	1	4	13	10	8	7
1 gal. (Plot 2)[b]	2	4	18	5	15	5
1 gal. (Plot 6)	3	5	25	15	19	15
Check (Plot 1)	6	2	[c]	10	9	6
Check (Plot 7)	2	5	5	3	9	7
Check (Plot 8)	0	2	[c]	8	5	5

[a] Nets cleared.
[b] Plot 2 was sprayed one day before other plots — each reading is one day more from treatment.
[c] No collections were made in Check (Plot 1) and Check (Plot 8) on May 20. This was done the following day and is an accumulative sample not to be compared to remainder of collection on May 21.

It will be noted that natural mortality during the period was fairly high. This continued into June according to other observations. On May 29 it was noted that the linden looper had reached the last two instars and a considerable amount of heavy feeding was expected over the next week. The weather continued cold and wet, and on June 2 it was estimated that the linden looper had become reduced in numbers. On these cold, wet days the loopers did not feed, but the immediate cause of this apparent reduction was an epizootic in this species. Dr. Frank Lewis, Northeastern Forest Experiment Station, kindly examined some diseased loopers and found a number of these larvae infected with either virus or a microsporidian.

Frass

Following the last collection of dead and paralyzed larvae on May 27, the drop nets were used to collect frass of the various species of caterpillars still feeding on the foliage above the nets. The frass of the different species or instars was not separated and weights of frass are a composite. After this date no more larval collections were made from the drop nets. The average dry weight of frass per net for all species is given in Table 6.

Analysis of the data for each collection date indicated that there was a significant difference at the 1 per cent level between treated and untreated areas on May 27 and 28. In none of the later collections was there a statistical

Table 6. Average weight in grams of frass collected per net from various species of caterpillars feeding in treated and untreated woods

Dosage rate	Date Collected				
per acre	May 27	May 28	June 3	June 6	June 17
¼ gal. (Plot 5)	1.28	.50	5.26	3.82	4.20
½ gal. (Plot 3)	.47	.25	3.01	2.71	3.41
½ gal. (Plot 4)	1.09	.64	7.66	5.92	6.53
1 gal. (Plot 2)	.72	.62	3.90	2.53	3.43
1 gal. (Plot 6)	.78	.37	5.21	3.97	7.17
Check (Plot 1)	3.39	1.73	9.19	4.50	7.84
Check (Plot 7)	2.12	.98	6.32	3.52	5.72
Check (Plot 8)	3.79	1.33	8.64	4.67	4.44
LSD 1%	1.24	.67			

difference between the treated and untreated plots. On June 3 the feeding in Plots 2 and 3 was less than the nearest control area, Plot 7, but an increase in frass was evident in Plots 4, 5, and 6. This general trend continued as indicated by the collections on June 6 and 17.

On the last date, the feeding in Plots 4 and 6 was not very different from the controls, but the feeding in Plot 5 appeared to have dropped. Feeding as indicated by frass, remained lower in Plots 2 and 3 than the check. The feeding in Plot 2 (1 gal./acre) was clearly lower, as noted by observations along the borders where there was a visible difference between treated and untreated woods. Observations along the borders of Plot 3, however, did not indicate any noticeable difference between treated and untreated woods. It appeared that the amount of feeding had generally decreased along all of the ridge on which Plot 3 was situated, and there was little damage or frass production.

Defoliation

Estimate of feeding

The results of the defoliation estimates are given in Table 7. By far the heaviest defoliation occurred in Plot 1. The estimate of 76 per cent is much higher than any of the other check areas counted. If the treated areas had been compared only to this plot it would have appeared that control of the caterpillar complex was quite good. However, when Plots 2 and 3 are compared to the nearest check (Plot 7), and Plots 4, 5, and 6 are compared with Plot 8 a more realistic estimate of defoliation is possible.

There was definite protection of the foliage in Plot 2 by treatment with 1 gallon of Thuricide 90T per acre. It was possible to see this difference clearly on the eastern border of the plot where defoliation was estimated at 25 per cent compared to 13 per cent in the plot.

Although defoliation in Plot 3 (½ gal. Thuricide/acre) was estimated at 31 per cent as compared to a check (Plot 7) with 38 per cent, there was probably no real difference due to treatment. Plot 3 lies just over a steep ridge on a slope that falls away to the west (Figure 1). Readings of defoliation in Plot 7 were made from the road west up on the ridge where this check lies northeast of Plot 3. When those readings made on the ridge in the vicinity of Plot 3 are averaged, an estimate of 32 per cent is obtained for untreated oaks or almost the same as the defoliation estimate in Plot 3.

Table 7. Estimated defoliation of oak trees in plots treated with various doses of *B. thuringiensis* and in untreated control plots

Dosage rate per acre	Defoliation per cent
¼ gal. (Plot 5)	33
½ gal. (Plot 3)	31
½ gal. (Plot 4)	27
1 gal. (Plot 2)	13
1 gal. (Plot 6)	32
Check (Plot 1)	76
Check (Plot 7)	38
Check (Plot 8)	35
Check (near Plot 2)	25

By contrast to Plot 2, no effect of treatment with 1 gallon of Thuricide could be noted in Plot 6. The estimate of defoliation was very close to that of Plot 8 (check), the nearest control plot. No difference between treated and untreated woods on the border of Plot 6 could be seen.

The estimate of defoliation in the oaks in Plot 4 appears slightly lower than that in Plot 8 (check), but here again this is probably not a real difference. We were unable to observe any effect from treatment along the southern and eastern borders of this plot. The apparent difference probably reflects the variability of the infestation of loopers and gypsy moths.

Light meter readings

Estimated defoliation in Plot 2 was rated at 13 per cent (Table 7) while a series of readings just outside Plot 2 along the eastern border gave an average estimate of 25 per cent. Although the numerical difference seems small, a walk from Plot 2 east into the untreated woods revealed a distinct difference in the amount of defoliation, particularly in the amount of sunlight coming through the canopy.

A Weston light meter was used to check these differences in light intensity. A series of readings were made in the treated plot, then in the untreated area, and finally back in the treated plot to account for changes in light intensity due to movement of the earth relative to the sun. Thirty-five readings beginning in the treated plot gave an average meter reading of 77. Twenty-two readings in nearby untreated woods gave a meter reading of 158 while a return to Plot 2 produced an average reading of 83 for 16 readings in different locations. Light penetration through the untreated canopy was roughly double that in the plot treated with the 1-gallon rate of Thuricide. This was the only plot where a clear line of demarcation was noted.

Parasites and Predators

No particular investigation of the parasites or predators was undertaken, but several miscellaneous observations were made. A considerable bird population, as described later, was present to prey on the caterpillars. Most of these birds (the cuckoo being an exception) would feed on the geometrid larvae rather than on the hairy gypsy moth larvae.

A large number of adult parasitic flies were noted in late May. A few were captured and sent to C. W. Sabrosky, Agricultural Research Service, Washington. His identifications were used as the basis to count the numbers and kinds of adult flies present. Those captured at that time were as follows:

3 *Gonis sagax* Tns. (host *Alsophila pometaria* acc. to Thompson 1944)
1 *Omotoma fumiferanae* (Tot.)
6 *Blepharipa scutellata* (R.D.) (wide host range)
37 *Pseudotachinomyia slossonae* (Tns.) (host *Alsophila pometaria* and *Erannis tilaria* acc. to Thompson 1944)
6 *Sarcophaga aldrichi* Parker (secondary invaders of hosts killed by ichneumonids acc. to Campbell 1963)

Collections made in June were examined for external evidence of attack by parasites. The results for specimens of *Erannis tilaria* are shown in Table 8.

Table 8. Larvae of *Erannis tilaria* on oak, beech, and birch showing external evidence of parasitic attack

Dosage rate per acre	Number	Per cent parasitized
¼ gal.	69	62.3
½ gal.	79	32.9
1 gal.	83	18.1
Check	166	45.8

The caterpillars in the plots receiving the heavy dosage of spray generally had a smaller percentage of their numbers showing external evidence of parasitic attack than those in the check or the plot receiving the lightest dosage.

Calosoma beetles were numerous and active in the latter part of May and early June, but no counts were made.

Effects On Wildlife

Effects on aquatic insects

The effects of the spray on aquatic insects was investigated in one of the plots (Plot 6) that received the heaviest dosage. The numbers and kinds of insects within the stream were checked by means of a Surber foot-square sampler. Ten foot-square samples were taken both before and after spraying at a point well within the sprayed area and, as a check, at another point upstream from the sprayed area. It is believed that this number of samples gives a fair qualitative measure of population changes at the generic level (Hitchcock 1960.)

Three categories of insects were considered. Those that were found in any number in the post-spray count were considered to be (1) "unaffected." Those insects found only in the pre-spray count but not later were considered to be either (2) "eliminated" if found in numbers of five or more in pre-spray counts or (3) "possibly eliminated" if found only in numbers less than five before treatment. Results are shown in Table 9.

Only three genera could be considered to be eliminated from the treated area. One of these, the caddisfly *Rhyacophila,* had also disappeared from the check area, probably because of the emergence of the adults. Only five specimens of the stonefly *Leuctra,* were found in the pretreatment counts, and its disappearance may represent an artifact of sampling size. Another stonefly, *Peltoperla,* was present as eight specimens in pretreatment counts. It was not found after spraying in the treated area.

As *Bacillus thuringiensis* has a relatively short effective life in water and must be eaten to kill an insect, it was initially believed that any deaths would be most likely to occur with those insects that strain the passing water for food. The blackfly larvae, *Simulium* and *Prosimulium,* obtain food in this manner but were collected after as well as before the spray was applied. The caddisfly larva, *Hydropsyche,* spins a net to catch passing food particles and as the Trichoptera are closely related to the Lepidoptera, it was thought to be potentially the most likely victim of the spray. Although *Hydropsyche* did not reappear in the postreatment counts, the population in pretreatment counts was too small to permit valid conclusions.

Table 9. Survival of insect genera one week after an aerial spray of *Bacillus thuringiensis,* based on 10 square-foot samples

Insect	Treated[a]	Untreated[a]	Insect	Treated	Untreated
Odonata			Diptera		
Cordulegaster	X	X	Chironomidae	X	X
Lanthus	X	X	*Hexatoma*	X	A
Agrion	..	A	*Holorusia*	X	..
Plecoptera			*Palpomyia*	X	A
Peltoperla	O	X	*Prosimulium*	X	X
Nemoura	X	X	*Simulium*	X	X
Alloperla	X	X	*Dicranota*	X	..
Acroneuria	X	X	*Cnephia*	A	..
Leuctra	O	X	*Tipula*	..	X
Megaloptera			Tabanidae	..	A
Sialis	X	X	Trichoptera		
Nigronia	X	A	*Pycnopsyche*	A	A
Coleoptera			*Ptilostomis*	A	A
Elmidae	X	O	*Hydropsyche*	A	X
Anchytarsus	X	X	*Psilotreta*	X	X
Ephemeroptera			*Hydroptila*	X	A
Ephemerella	X	X	*Neophylax*	A	A
(*Eurylophella*)			*Rhyacophila*	O	A
Habrophlebia	X	X	*Oecetis*	X	..
Paraleptophlebia	A	A	*Lepidostoma*	X	..
Leptophlebia	A	..	*Diplectrona*	..	X
Stenonema	X	..	*Asellus*	X	X

[a] X, found after spraying; A, found only in pretreatment counts and in numbers less than 5; O, found only in pretreatment counts but in numbers of 5 or more.

In summary, the treated plot showed 24 genera surviving, 7 possibly eliminated, and 3 eliminated. The check showed 19 genera surviving, 11 possibly eliminated, and 1 eliminated. These differences are not considered to be significant, and the tentative conclusion was that there was no effect of the spray on the aquatic insects present. These results may be compared with the sampling after a DDT spray on similar streams in Connecticut by Hitchcock (1960).

Effects on birds and small mammals

Through the courtesy of the Connecticut Department of Fish and Game and the U. S. Fish and Wildlife Service, surveys were made by game biologists Ruth Billard and Norman Holgersen to determine the effects of the spray on the population of certain vertebrate animals.

Their results in snap-trapping for small rodents is summarized in Table 10. The spray had no appreciable effects on these animals. Indeed, all plots showed a greater number trapped after spraying than before except the most lightly treated plot. The total on one of the most heavily treated plots was quite similar to that of the check plot, and it may be safely concluded that the spray caused no immediate effect on the population of these mammals.

The Hartford Bird Study Club and Miss Billard kindly consented to make a bird census of the plots. These results are shown in Table 11. Both the heavy and the medium dosages showed one plot with a considerable population in

Table 10. Results of snap-trapping for small mammals in area sprayed with *Bacillus thuringiensis*[a]

Amount per acre	Date	Trap nights	White-footed mouse	Red-backed mouse	Woodland jumping mouse	Meadow mouse	Total
1 gal.	7-9[b]	150	2	3	1	0	6
(Plot 2)	20-23	200	9 (*4*[c])	1 (*1*)	0	0	10
1 gal.	7-9[b]	120	2	1	0	0	3
(Plot 6)	20-23	200	10 (*6*)	2 (*1*)	1	0	13
½ gal.	7-9[b]	150	4	1	0	0	5
(Plot 4)	20-23	120	6 (*4*)	0	0	0	6
¼ gal.	7-9[b]	120	4	0	0	0	4
(Plot 5)	20-23	160	4 (*2*)	1	0	0	5
Check	7-9[b]	150	3	2	0	0	5
(Plot 1)	20-23	200	7 (*4*)	6	1	1	15

[a] Data from R. Billard and N. Holgersen.
[b] Pretreatment trapping abandoned after third day because of the number of songbirds being taken.
[c] Figures in italics show number of immature mice trapped.

species and individuals and the other plot with relatively few. Even though the check plot showed a slightly greater number of species, no discernible pattern appeared in the dosage series, and it may be concluded that the spray exhibited no immediate effect on the resident bird population.

DISCUSSION

There were two main points of interest in this experiment. During the winter of 1962-63 a new liquid suspension called Thuricide 90T was developed in the hope that this formulation would reduce some of the problems of spray application by aircraft. Much is known about ground application of *B. thuringiensis* formulations and the literature is too large to include here. A series of field studies using aircraft have given valuable information on handling and effectiveness of wettable powder and on problems of sampling (Lewis et al. 1961, 1962, and Cantwell et al. 1961). However, the unsatisfactory physical characteristics of wettable powders have hindered the accurate assessment of gypsy moth control by aircraft.

The second point was how effective this *B. thuringiensis* formulation was against the gypsy moth and a complex of loopers also present throughout the experimental area. In the past few years loopers have been very important woodland defoliators, often appearing in conjunction with the gypsy moth. It is known that sprays of *B. thuringiensis* applied by ground equipment are effective against some of these loopers, but less is known about their effectiveness when applied by aircraft. In laboratory tests Thuricide 90T gave good indication of its ability to kill gypsy moth larvae.

Counts were made on the abundance of loopers and gypsy moths present in the experimental plots. The number of early spring egg masses of the gypsy moth showed a decline toward the north end of the test area, but all plots contained a considerable number of eggs. A moderately heavy population of

Table 11. Birds observed in treated and untreated plots

Species	Check (Plot 1)	Amount of *Bacillus thuringiensis* applied per acre 1 gal. (Plot 2)	½ gal. (Plot 3)	½ gal. (Plot 4)	¼ gal. (Plot 5)	1 gal. (Plot 6)
Cuckoo	5	..	..	..	1	..
Flicker	..	1	1	..	..	2
Hairy Woodpecker	1	..	..	..	..	4
Downy Woodpecker	2	..	..	2	..	..
Crested Flycatcher	..	..	..	2	..	4
Wood Pewee	..	..	..	4	..	..
Blue Jay	3	1	3	2	5	3
Crow	2	..	..	..	..	..
Chickadee	2	..	1	..	..	5
Tufted Titmouse	..	..	..	..	3	..
White-breasted Nuthatch	2	1	..	2	..	4
Red-breasted Nuthatch	..	..	..	..	..	1
House Wren	..	1	..	..	..	..
Catbird	..	..	..	1	1	..
Wood Thrush	3	6	2	12	7	11
Olive-backed Thrush	1	..	..	..	..	..
Veery	..	..	..	4	..	5
Yellow-throated Vireo	1	..	..	..	..	..
Red-eyed Vireo	2	..	..	8	..	3
Black-and-white Warbler	3	2	2	1	1	..
Worm-eating Warbler	..	..	..	3	..	..
Parula Warbler	1	..	..	..	..	..
Black-throated Green Warbler	2	..	..	..	..	..
Blackpoll Warbler	1	..	..	..	..	..
Ovenbird	10	7	5	20	10	17
Northern Yellowthroat	..	..	..	..	..	1
Redstart	1	..	..	..	..	..
Baltimore Oriole	..	..	..	10	..	..
Bronzed Grackle	..	..	..	2	..	..
Cowbird	1	..	..	4	..	..
Scarlet Tanager	7	1	..	4	2	9
Rose-breasted Grosbeak	4	2	..	2	..	2
Goldfinch	..	..	..	..	..	2
Towhee	9	10	10	9	3	11
Unidentified Warblers	6	..	..	4	..	5
Unidentified Species	13	2	7	13	15	17
TOTAL SPECIES	21	10	7	18	9	16
TOTAL INDIVIDUALS	82	34	31	109	48	106

Note: Because approximately 75 per cent of the birds counted were singing, resident males, it may be assumed that a certain number of silent females were in the vicinity. Data from the Hartford Bird Study Club and R. Billard.

geometrids was expected but could not be measured until after the eggs had hatched. Larval counts before spraying showed a surprising uniformity among gypsy moth larvae and to a lesser degree among geometrid larvae. Examination of egg masses at this time showed that the gypsy moth hatch was complete. The linden looper (*Erannis tilaria*) and the geometrid *Phigalia titea* were most abundant while lesser numbers of an unidentified looper occurred. *Argyrotoza semipurpurana,* an oak leaf roller, was also common. Males of the spring cankerworm (*Paleacrita vernata*) were extremely abundant earlier but for some reason an infestation never developed and larvae were rare.

The importance of having untreated control plots beside or near each treated plot was clearly demonstrated in these tests. Before treatment two extra check plots (Fig. 1) were established in addition to the original control plot (Plot 1): one near Plots 2 and 3, and one near Plots 4 and 6. Despite the relative uniformity of the original population and geographical proximity of the plots there were marked differences in defoliation between plots treated the same. The heaviest defoliation in the area eventually occurred in and near Plot 1. The degree of defoliation in the remaining checks was much lower.

Thuricide 90T at the high dose was quite uneven in its effect on the caterpillars feeding on treated leaves. We have no clear explanation for its variability in protecting the foliage in one plot and not in the other. Probably more than one factor influenced the outcome and a few may be considered here.

Measurements of head capsule size strengthened general observations that larvae did not refuse the treated foliage. There was some evidence that *B. thuringiensis* was less effective against *Phigalia titea* than the other geometrids and this aspect will be investigated further. There was also some indication that the unidentified looper hatched later than the other geometrids.

Cool weather had a considerable influence on the normal development of the larvae. The first 10 days of May were warm and at the end of this period the eggs were hatched, and the larvae were feeding vigorously. The weather then became progressively cooler until the end of the month, and on many of these days the larvae did little or no feeding. Control could be very uneven if the larvae did not feed well during the 5 days following application of *B. thuringiensis* since the residual life of this material is short.

An epizootic caused by a microsporidian and by virus disease depleted the number of *E. tilaria* toward the end of May before their development was complete. Although this disease appeared too late to affect the outcome of the test, it may have had some effect on the amount of feeding and defoliation in untreated plots.

The reasons for reduced incidence of parasitic attack on linden looper in the plots with heavier doses of the insecticide (Table 8) are unknown. It would seem unlikely that the material either killed or repelled the parasites. A possible explanation might be that parasitized individuals succumbed to the *Bacillus* and thus were not present for the later counts.

Treatment with 1 gallon of Thuricide per acre controlled defoliation rather well in Plot 2. There was little evidence of protection in Plot 6 treated with 1 gallon per acre and in the other plots sprayed with lower dosages.

Thuricide appeared to cause negligible damage to aquatic insects and vertebrate wildlife.

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